

Figure 9

151

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mus HGYFLPGQFA FSRALRTK

rat RIYFFPGQFA FSRAL---

tig2 HSFYFPGQFA FSKALPRS

sus HSYYFPGQFA FFKALPPS

bos HSYYLPGQFA FIKAL---

gallus DVLYLPGMFA FSKGLP~~

Identities :

	bos.pep	mus.pep	sus.pep	gallus	rat.pep
tig2.pep	83.750	56.250	86.503	30.675	61.392
bos.pep		54.375	87.500	31.875	56.329
mus.pep			54.375	31.677	73.125
sus.pep				31.288	58.228
gallus.pep					30.818

Figure 10 Primary screening of HPLC fractions obtained from the fractionation of human ovary ascites.

The different fractions obtained following fractionation of human ovary ascites were diluted fivefold in the buffer assay and tested in aequorin assay using a cell line expressing ChemR23 (open circles) or cell lines expressing not related receptors (closed triangles and squares). The response obtained for each fraction was normalized using the ATP response of each cell line.

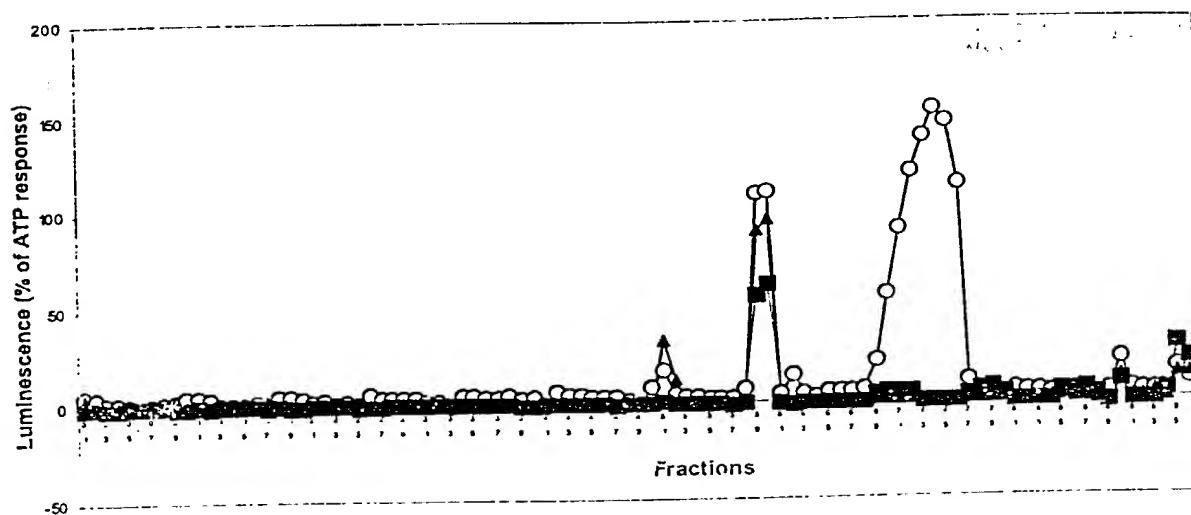


Figure 11 Activation of ChemR23 by cells transfected with TIG2

293 T cells were transiently transfected with pCDNA3- TIG2 or with pCDNA3 alone (mock transfected). Increasing volumes of the supernatant collected 4 days following transfection were analysed in a aequorin-based assay with CHO cells expressing ChemR23. A representative experiment is shown. Assay was performed in triplicate and SD are indicated.

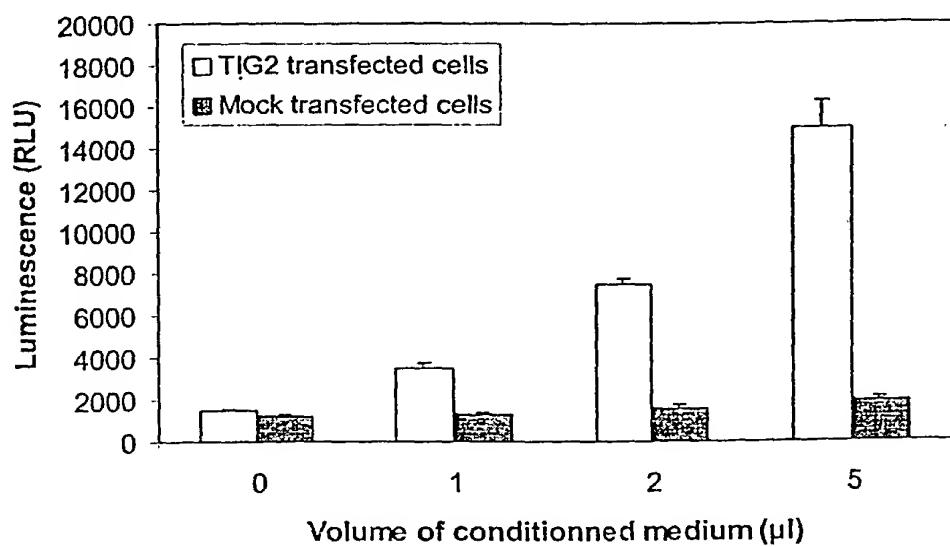


Figure 12 Characterization of antibodies directed against ChemR23
A mixture of recombinant cells made up of 2/3 recombinant ChemR23 CHO cells and 1/3 recombinant HCR CHO cells (negative control) was subject to react with either a supernatant of the anti ChemR23 5C 1H2 monoclonal antibody (thick line) or a supernatant with no known antibody activity (thin line, grey filling). After staining with FITC labeled anti mouse Ig these preparations were analysed by flow cytofluorometry. Results are displayed as a histogram of the number of cells (Events axis) expressing a given fluorescence (FL1-H axis). Monoclonal 5C 1H2 allowed to discriminate the ChemR23 recombinant sub-population of cells from the negative control cells as evidenced by the relative proportions of both type of cells. The background fluorescence of the assay is given by the second staining (grey filling).

